

WHAT IS CLAIMED IS:

1. A method for making a polypeptide in the form of inclusion bodies, said method comprising

5 (a) obtaining a host cell comprising a first nucleic acid molecule encoding said polypeptide operatively linked to a second nucleic acid molecule encoding an inclusion partner protein thereby forming a gene fusion construct; and
(b) cultivating said host cell under conditions favoring production of said polypeptide as inclusion bodies in said host cell.

2. The method of claim 1, further comprising

10 (c) isolating said inclusion bodies from said host cell; and
(d) releasing said polypeptide from said inclusion bodies.

3. The method of claim 1, wherein said first nucleic acid molecule is obtained from a bacterial cell.

15 4. The method of claim 1, wherein said first nucleic acid molecule is obtained from an animal cell, a plant cell or a yeast cell.

5. The method of claim 4, wherein said animal cell is a mammalian cell.

6. The method of claim 5, wherein said mammalian cell is a human cell.

20 7. The method of claim 1, wherein said second nucleic acid molecule is obtained from a bacterial cell.

8. The method of claim 7, wherein said bacterial cell is an *Escherichia coli* cell.

9. The method of claim 1, wherein said inclusion partner protein is thioredoxin or a modified thioredoxin.

5 10. The method of claim 9, wherein said modified thioredoxin is a truncated thioredoxin.

10 11. The method of claim 10, wherein said truncated thioredoxin is a carboxy terminal-truncated form of *Escherichia coli* thioredoxin which is encoded by a nucleic acid molecule having a nucleotide sequence as set forth in SEQ ID NO:8.

12. The method of claim 1, wherein said host cell is a bacterial cell.

13. The method of claim 12, wherein said bacterial cell is an *Escherichia coli* cell.

15 14. The method of claim 1, wherein said polypeptide is toxic to said host cell.

15. The method of claim 1, wherein said polypeptide is a fragment of the gene 32 protein of bacteriophage T4, a fragment of *KpnI* methylase, a fragment of *Escherichia coli* Dead-Box protein, or a fragment of *Escherichia coli* thioredoxin protein.

16. The method of claim 15, wherein said fragment of the gene 32 protein of bacteriophage T4 is encoded by a nucleic acid molecule having a nucleotide sequence as set forth in SEQ ID NO:1.

5 17. The method of claim 15, wherein said fragment of *KpnI* methylase is encoded by a nucleic acid molecule having a nucleotide sequence as set forth in SEQ ID NO:17.

18. The method of claim 15, wherein said fragment of *Escherichia coli* Dead-Box protein is encoded by a nucleic acid molecule having a nucleotide sequence as set forth in SEQ ID NO:14.

10 19. The method of claim 15, wherein said fragment of *Escherichia coli* thioredoxin protein is encoded by a nucleic acid molecule having a nucleotide sequence as set forth in SEQ ID NO:11.

20. The method of claim 1, wherein said gene fusion construct comprises pTrcprl-monomer or pTrxA-concat.

15 21. A vector comprising a gene fusion construct in which a first nucleic acid molecule encoding a polypeptide is operably linked to a second nucleic acid molecule encoding an inclusion partner protein, wherein when said gene fusion construct is introduced into a host cell said host cell produces said polypeptide as inclusion bodies.

20 22. The vector of claim 21, wherein said inclusion partner protein is thioredoxin or a modified thioredoxin.

23. The vector of claim 22, wherein said modified thioredoxin is a truncated thioredoxin.

24. The vector of claim 23, wherein said truncated thioredoxin is a carboxy terminal-truncated form of *Escherichia coli* thioredoxin which is encoded by a nucleic acid molecule having a nucleotide sequence as set forth in SEQ ID NO:8.

25. The vector of claim 21, wherein said vector is an expression vector.

26. The vector of claim 25, wherein said expression vector is pTrc99A or pTrxfus.

27. A host cell comprising the vector of claim 21.

28. The host cell of claim 27, wherein said host cell is a bacterial cell.

29. The host cell of claim 28, wherein said bacterial cell is an *Escherichia coli* cell.

30. A method for producing a polypeptide comprising culturing the host cell of claim 27 under conditions favoring the production of said polypeptide as inclusion bodies in said host cell.

31. The method of claim 30, further comprising

- (b) isolating said inclusion bodies from said host cell; and
- (c) releasing said polypeptide from said inclusion bodies.

32. The method of claim 2 or claim 31, wherein said releasing of said polypeptide from said inclusion bodies is accomplished by a chemical cleavage method.

5 33. The method of claim 2 or claim 31, wherein said releasing of said polypeptide from said inclusion bodies is accomplished by an enzymatic cleavage method.

10 34. The method of claim 32, wherein said chemical cleavage method comprises contacting said inclusion bodies with a polypeptide-releasing amount of a chemical cleavage agent under conditions favoring the release of said polypeptide from said inclusion bodies.

35. The method of claim 34, wherein said chemical cleavage agent is cyanogen bromide or hydroxylamine.

15 36. The method of claim 33, wherein said enzymatic cleavage method comprises contacting said inclusion bodies with a polypeptide-releasing amount of a enzymatic cleavage agent under conditions favoring the release of said polypeptide from said inclusion bodies.

37. The method of claim 36, wherein said enzymatic cleavage agent is factor Xa, thrombin or enterokinase.

20 38. The method of claim 37, wherein said enzymatic cleavage agent is enterokinase.

39. A polypeptide produced by the method of claim 1 or claim 30.

40. The plasmid pTrcpri-monomer.

41. The plasmid pTrxA-concat.

42. A method for making a protein molecular weight ladder composition comprising a plurality of polypeptides of differing molecular weights, said method comprising

(a) obtaining one or more nucleic acid molecules wherein each of said nucleic acid molecules encodes one or more polypeptides of different molecular weights of said molecular weight ladder;

(b) transforming one or more host cells with one or more of said nucleic acid molecules;

(c) culturing said host cells under conditions favoring the production of each of said polypeptides of said molecular weight ladder; and

(d) isolating each of said polypeptides.

43. The method of claim 42, wherein at least one of said nucleic acid molecules encodes a plurality of said polypeptides of different molecular weights of said molecular weight ladder.

44. The method of claim 42, wherein said nucleic acid molecules each encode a different polypeptide of said molecular weight ladder.

45. The method of claim 43, wherein said host cell comprises said nucleic acid molecule encoding said plurality of polypeptides of said molecular weight ladder.

46. The method of claim 44, wherein each of said host cells comprises a different nucleic acid molecule each encoding a different polypeptide of said molecular weight ladder.

5 47. The method of claim 44, wherein said host cell comprises two or more of said nucleic acid molecules each encoding a different polypeptide of said molecular weight ladder.

48. The method of claim 46, wherein said method further comprises admixing each of said different polypeptides to form a molecular weight ladder.

10 49. The method of claim 42, wherein said polypeptides are produced as inclusion bodies.

50. The method of claim 42, wherein said nucleic acid molecule is inserted into a vector prior to transforming said host cells.

51. The method of claim 50, wherein said vector is an expression vector.

15 52. A method of making one or more stained polypeptides, comprising incubating said polypeptides with one or more protein-binding dyes under incubation conditions sufficient to substantially completely complex said polypeptides with said dyes.

20 53. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes in a solution having a pH of about 7 to about 12.

54. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes in a solution having a pH of about 7 to about 10.

5 55. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes in a solution having a pH of about 8 to about 10.

56. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes at a temperature of about 20°C to about 90°C.

10 57. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes at a temperature of about 40°C to about 80°C.

15 58. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes at a temperature of about 45°C to about 70°C.

59. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes at a temperature of about 50°C.

20 60. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes for about 4 to about 200 hours.

61. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes for about 12 to about 24 hours.

5 62. The method of claim 52, wherein said incubation conditions comprise incubating said polypeptides with said one or more dyes for about 12 to about 18 hours, or overnight.

63. The method of claim 52, wherein said polypeptides provide a molecular weight ladder composition.

10 64. The method of claim 63, wherein said molecular weight ladder composition comprises a first polypeptide stained with a first protein-staining dye and a plurality of polypeptides of differing molecular weights from said first polypeptide and from each other stained with a second protein-staining dye.

15 65. The method of claim 64, wherein said first protein-staining dye is selected from the group consisting of remazol brilliant blue R, eosin isothiocyanate, and malachite green isothiocyanate.

66. The method of claim 64, wherein said first protein-staining dye is eosin isothiocyanate.

67. A stained protein made according to the method of claim 52.

20 68. A stained protein molecular weight ladder composition made according to the method of claim 63.